Effect on K_{ATP} Channel Activation Properties and Tissue Selectivity of the Nature of the Substituent in the 7- and the 3-Position of 4*H*-1,2,4-Benzothiadiazine 1,1-Dioxides

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Received December 11, 2003

The present work explored 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides diversely substituted in the 7-position. Those compounds, structurally related to previously described potassium channel openers such as the benzothiadiazine dioxide BPDZ 73, were tested as putative K_{ATP} channel activators on the pancreatic endocrine tissue and on the vascular smooth muscle tissue. The nature of the substituent introduced in the 7-position as well as the nature of the alkylamino side chain in the 3-position strongly affected both potency and tissue selectivity of 4*H*-1,2,4-benzothiadiazine 1,1-dioxides. Thus, compounds bearing in the 7-position a methyl or a methoxy group or devoid of a substituent in this position, and bearing an ethyl, an isopropyl, or a cyclobutylamino group in the 3-position were found to be potent and selective inhibitors of insulin release from rat pancreatic B-cells (i.e. **10a**, **10b**, **12b**, **12d**, **22c**). In contrast, 3-alkylamino-7-trifluoromethyl- (**20a**−**c**) and 3-alkylamino-7-pentyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**11a**,**b**) expressed a marked myorelaxant activity on rat aorta ring. Among the latter compounds, the 3-alkylamino-7-pentyl derivative (**11a**) showed a clear selectivity for the vascular smooth muscle tissue. The present work gives new insights into the role of the substituent in both the 7- and the 3-position for the design of 4*H*-1,2,4-benzothiadiazine 1,1-dioxide potassium channel openers exhibiting different tissue selectivity profiles.

Introduction

ATP-sensitive potassium channels (K_{ATP} channels) are involved in the transmembrane movement of potassium ions and the control of the cell membrane potential.[1] These channels are able to link the membrane excitability to the metabolic state of the cell. They have been characterized in numerous cell types such as cardiac, pancreatic B (insulin-secreting)-cells, skeletal muscles cells, smooth muscle cells, and central neurons.[2−8] Over the past few years, an increasing interest in drugs (diazoxide (**1**), pinacidil (**2**), cromakalim (**3**), etc.) activating K_{ATP} channels has been observed (Figure 1). Such compounds, named potassium channel openers (PCOs), mainly exert their biological effects by promoting membrane hyperpolarization.[9−11] They are able to interfere with several physiological processes such as insulin release from pancreatic B-cells and contractile activity from smooth muscle cells.[12,13] The therapeutic interest of potassium channel openers is of significance since they provide efficient tools to interfere with the cell excitability. Clinical studies have shown that short-term treatment with the K_{ATP} channel activator diazoxide preserved endogenous insulin production in patients with newly manifested autoimmune diabetes and reduce body fat in hyperinsulinemic obese adults.[14,15] However, the poor tissue selectivity of diazoxide impairs its clinical use due to marked side effects such as hypertrichosis, edema, and headache.[16] K_{ATP} channels are large heteromultimers composed of two different proteinic subunits coassembled in a 4:4 stoichiometry to form an octameric channel.[17] One subunit, the Kir6.x, is a traditional channel protein belonging to the inward rectifying potassium channel superfamily (Kir) while the second subunit refers to the sulfonylurea receptor (SUR). Tissue selectivity properties of PCOs are correlated to the identity of the SUR isoform expressed in the tissue. For instance, the Kir6.2/SUR1 channel, which is found in neuronal cells and pancreatic B-cells, is activated by diazoxide but only slightly by pinacidil.[9,18] The Kir6.2/SUR2A channel, predominant in skeletal cardiac muscle cells, is activated by pinacidil but not by diazoxide while the Kir6.2/SUR2B channel,

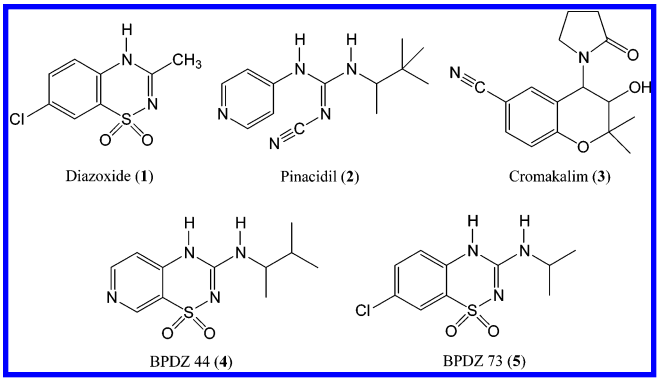

Figure 1. Chemical structure of several K_{ATP} channel openers.

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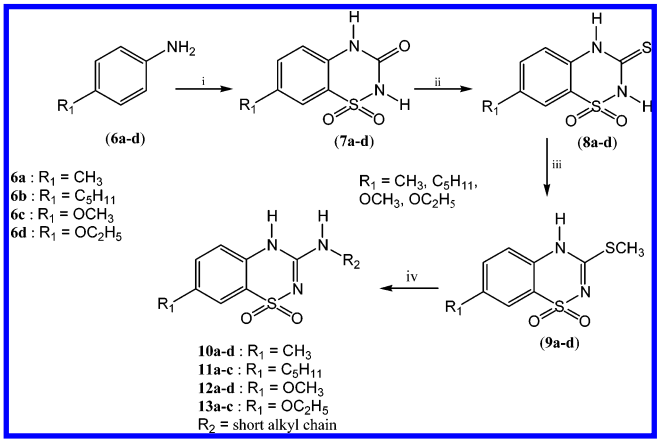
10.1021/jm0311339 CCC: $30.25
Published on Web 04/22/2005

Scheme 1[a]



[a] Reagents and conditions: (i) 1: $ClSO_2NCO$, CH_3NO_2; 2: $AlCl_3$; (ii) P_2S_5, pyridine, Δ; (iii) $NaHCO_3$, CH_3I, CH_3OH/H_2O; (iv) R_2NH_2, Δ.

found in smooth muscle cells, is activated both by pinacidil and diazoxide.[19−22] Thus, and due to the ubiquitous distribution of K_{ATP} channels, the development of novel PCOs should be linked to the expression of a high selectivity for a single channel subtype located on a single target tissue.

Previous works performed in our laboratories led to the development of original 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides, among which BPDZ 44 (**4**) was reported to be a more potent and more selective pancreatic B-cell K_{ATP} channel opener than diazoxide (Figure 1).[23,24] To improve activity and selectivity for the pancreatic B-cell K_{ATP} channel, we also prepared the 7-halobenzenic counterparts of pyridothiadiazines. From those original compounds, BPDZ 73 (**5**) (7-chloro-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide, Figure 1) appeared to be one of the most potent pancreatic K_{ATP} channel activator reported to date.[12,25,26]

In the present study, we examined different series of 4*H*-1,2,4-benzothiadiazine 1,1-dioxides structurally related to BPDZ 73 (**5**). Particular attention was paid to the influence of the nature of the substituent in the 7-position (steric, lipophilic, electronic impact) and the size as well as the branching of the alkylamino side chain in the 3-position. The new compounds were examined as putative potassium channel openers on rat pancreatic islets and on rat aorta rings. The structure−activity relationships of these putative PCOs will also be discussed.

Chemistry

The synthetic pathways used to prepare the different 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides reported here are illustrated in Schemes 1−4.

The strategy described in Scheme 1 was based on previously reported synthetic procedures.[26] 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides bearing a methyl (**10a**−**d**), a pentyl (**11a**−**d**), a methoxy (**12a**−**c**), and an ethoxy group (**13a**−**c**) in the 7-position were obtained in a four-step reaction starting from the appropriate para-substituted aniline (**6a**−**d**). First of all, the anilines were transformed after reaction with chlorosulfonyl isocyanate into the corresponding 7-substituted 3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (**7a**−**d**).[27] Subsequent thionation of the oxo derivatives (**7a**−**d**) with phosphorus pentasulfide in pyridine led to 7-substituted 3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides (**8a**−**d**). In the next step, the synthesis of 7-substituted 3-(methylsulfanyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**9a**−**d**)[28] was achieved, with an excellent yield, by reaction of the thioxo derivatives (**8a**−**d**) with methyl iodide in the presence of sodium hydrogenocarbonate. Finally, the different 7-substituted 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**10a**−**d**, **11a**−**d**, **12a**−**c**, **13a**−**c**) were obtained by heating the methylsulfanyl intermediates (**9a**−**d**) in a sealed vessel with an excess of the appropriate alkylamine.

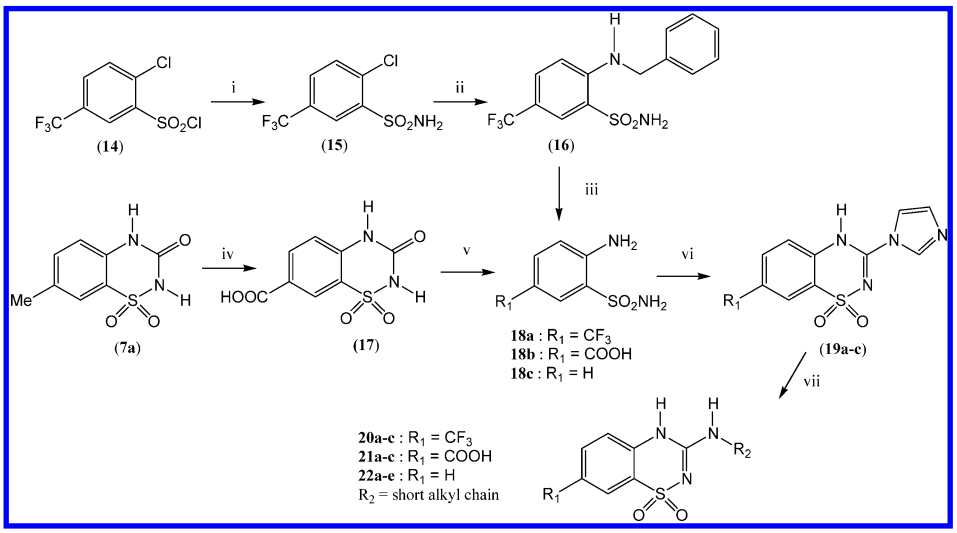
2-Amino-5-trifluoromethylbenzenesulfonamide (**18a**) (Scheme 2) was prepared from commercially available 2-chloro-5-trifluoromethylbenzenesulfonyl chloride (**14**). The latter intermediate (**14**), by reaction with a diluted solution of ammonia, gave access to 2-chloro-5-trifluoromethylbenzenesulfonamide (**15**). Nucleophilic substitution of the chlorine atom was achieved by reaction of **15** with benzylamine to form the intermediate **16**. Removal of the benzyl group by catalytic hydrogenolysis led to the desired key intermediate 2-amino-5-trifluoromethylbenzenesulfonamide (**18a**). The 4-amino-3-sulfamoylbenzoic acid synthesis (Scheme 2) started from 7-methyl-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7a**) which was oxidized by means of potassium permanganate to the corresponding acid (**17**). Subsequent hydrolysis of **17** under acidic conditions led to the desired compound **18b**.

The ring closure reaction between 5-substituted 2-aminobenzenesulfonamides and 1,1′-thiocarbonyldiimidazole may give access, according to the starting materials and the experimental conditions used, either to 7-substituted 3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides or to 3-(1*H*-imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**19a**−**c**).[29] In the case of 2-aminobenzenesulfonamides (**18a**−**c**), reaction with an excess of 1,1′-thiocarbonyldiimidazole in dioxane gave access to the 3-(1*H*-imidazol-1-yl)-substituted intermediates. The latter were converted, with good yields, to the corresponding 7-substituted 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**20a**−**c**, **21a**−**c**, **22a**−**e**) after reaction with the appropriate alkylamine.

As described in Scheme 3a, 3-alkylamino-7-cyano-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**24a**,**b**) were obtained from the substitution of the iodine atom of the corresponding 3-alkylamino-7-iodo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**23a**,**b**) [26] with cyanide ion as the cuprous salt in hot DMF. Compound **25** was obtained by reaction of **21b** with MeOH in the presence of H_2SO_4 whereas compound **26** resulted from the reaction of **21b** with carbonyldiimidazole in DMF followed by reaction of the activated intermediate with ammonia (Scheme 3b).

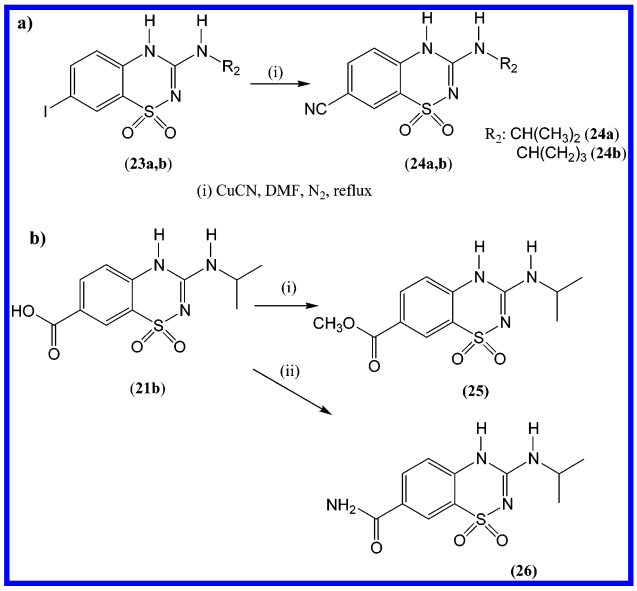
3-Alkylamino-7-methylsulfonyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**31a**−**c**) were not obtained from the ring closure reaction of 2-amino-5-methylsulfonylbenzenesulfonamide (**29**) with 1,1′-thiocarbonyldiimidazole and subsequent reaction with an appropriate alkylamine due to low yields of the imidazolyl intermediate obtained in that case. An alternative route in a four-step reaction, starting from 4-chloro-1-methylsulfonylbenzene (**29**), was used to gave access to the desired

Scheme 2[a]



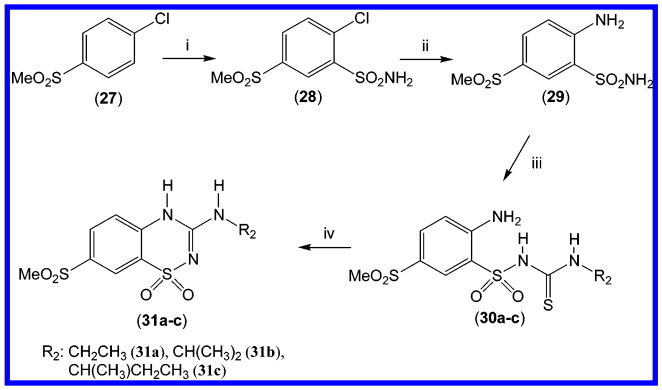
[a] Reagents and conditions: (i) NH_4OH; (ii) benzylamine, Δ; (iii) Pd/C 5%, H_2 5 bars, MeOH; (iv) $KMnO_4$; (v) H_2SO_4 50%, Δ; (vi) 1,1′-thiocarbonyldiimazole, dioxane, DMF, Δ; (vii) R_2NH_2, Δ.

Scheme 3[a]



[a] Reagents and conditions: (i) MeOH, H_2SO_4; (ii) 1: carbonyldiimidazole; 2: NH_4OH.

Scheme 4[a]



[a] Reagents and conditions: (i) 1: $ClSO_3H$, 2: NH_4OH; (ii) concd NH_4OH, Δ; (iii) R_2NCS, K_2CO_3, acetone; (iv) $N(C_2H_5)_3$, Cl_2CO, THF.

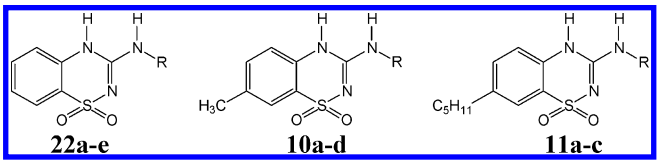
compounds (Scheme 4). Compound **28** was obtained from the reaction of 4-chloro-1-methylsulfonylbenzene (**27**) with chlorosulfonic acid, followed by the reaction of the sulfonyl chloride intermediate with aqueous ammonia. Treatment of **28** with a saturated aqueous solution of ammonia in a sealed vessel gave 2-amino-5-methylsulfonylbenzenesulfonamide (**29**). The latter was converted into the *N*-alkyl-*N*′-(2-amino-5-methylsulfonylbenzenesulfonyl)-thioureas (**30a**−**c**) after reaction with the appropriate alkylisothiocyanates. The sulfonylthioureas were converted by ring closure into the corresponding 3-alkylamino-7-methylsulfonyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**31a**−**c**) by treatment with a toluenic solution of phosgene in the presence of triethylamine at 0 °C.[30]

Results and Discussion

The ability of the newly synthesized compounds to inhibit the glucose-induced insulin secretion was evaluated on isolated rat pancreatic islets. Tables 1−4 report the in vitro results expressed as the percentage of residual insulin release at different drug concentrations. The data revealed that several drugs markedly inhibited the insulin releasing process. Moreover, some newly synthesized derivatives (**10a**−**d**, **12a**−**d**, **20b**, and **22a**−**e**) were found to be more potent than diazoxide used as a reference PCO.[23]

The nature of the substituent in the 7-position and in the 3-position strongly affected the activity on insulin secreting cells. 7-COOH-substituted compounds (**21a**−**c**) appeared to be completely inactive (Table 3). It is expected that the carboxylic group in the 7-position will be deprotonated at physiological pH (7.4), giving an anionic site in this position. Such an event could maybe explain the lack of biological activity. However, even when the carboxylic group was converted into the nonionic methyl ester group (**25**), the activity on B-cells remained to be negligible. Surprisingly, a carboxamido group (**26**) conferred a better activity. 7-Pentyl- (**11a**−**c**) (Table 1), 7-ethoxy- (**13a**,**c**) (Table 2), 7-cyano- (**24a**,**b**) (Table 3), 7-methylsulfonyl- (**31a**−**c**) (Table 3), 7-nitro- (**32a**,**c**−**e**)[31] (Table 4), 7-amino- (**33**)[31] (Table 4) and 7-acetamido- (**34**)[31] (Table 4) substituted 4*H*-1,2,4-benzothiadiazine 1,1-dioxide were also found to express a weak activity as inhibitors of insulin release. The

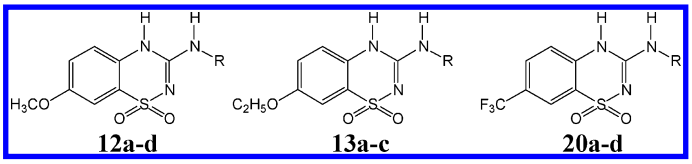
Table 1. Effects of 7-Substituted 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and Contractile Activity of K^+-Depolarized Rat Aorta Rings



		rat pancreatic B-cells % RIS[a]			
compd	R	50 μM	10 μM	1 μM	rat aorta rings ED_{50}[b] (μM)
22a	CH_2CH_3	N.D.	25.8 ± 2.0 (12)	77.4 ± 3.8 (23)	>300 (4)
22b	$CH_2CH_2CH_3$	N.D.	54.2 ± 3.2 (21)	93.8 ± 4.6 (16)	>300 (4)
22c	$CH(CH_3)_2$	N.D.	34.9 ± 2.0 (16)	73.7 ± 5.2 (15)	>300 (4)
22d	$CH(CH_3)CH_2CH_3$	N.D.	42.7 ± 1.5 (16)	90.4 ± 5.0 (16)	precipitate
22e	$CH(CH_2)_3$[d]	N.D.	29.8 ± 1.6 (14)	79.9 ± 4.5 (16)	precipitate
10a	CH_2CH_3	N.D.	8.6 ± 0.9 (12)	83.8 ± 4.2 (23)	210.2 ± 32.2 (6)
10b	$CH(CH_3)_2$	3.5 ± 0.3 (13)	8.5 ± 0.7 (14)	71.3 ± 3.5 (15)	200.0 ± 47.6 (3)
10c	$CH(CH_3)CH_2CH_3$	N.D.	50.7 ± 3.1 (23)	93.6 ± 5.9 (22)	15.5 ± 3.2 (10)
10d	$CH(CH_2)_3$[d]	3.7 ± 0.3 (11)	19.7 ± 1.3 (24)	99.5 ± 4.7 (23)	58.4 ± 7.0 (4)
11a	CH_2CH_3	N.D.	104.6 ± 5.2 (16)	N.D.	6.6 ± 0.3 (4)
11b	$CH(CH_3)_2$	N.D.	106.0 ± 5.3 (16)	N.D.	20.1 ± 4.6 (4)
11c	$CH(CH_2)_3$[d]	N.D.	95.3 ± 6.1 (15)	N.D.	>30 (4)
	diazoxide	26.7 ± 1.6 (16)[c]	73.9 ± 4.4 (16)[c]	87.5 ± 5.0 (15)[c]	22.4 ± 2.1 (11)[c]
	BPDZ 44	8.6 ± 0.9 (23)	13.7 ± 1.2 (23)	70.5 ± 4.4 (19)	154.4 ± 14.5 (8)[c]
	BPDZ 73	5.7 ± 0.5 (35)[c]	4.9 ± 0.4 (32)[c]	36.2 ± 2.4 (31)[c]	36.3 ± 2.2 (6)[c]

[a] RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [c] Published results (ref 26). [d] Cyclobutyl.

Table 2. Effects of 7-Substituted 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and Contractile Activity of K^+-Depolarized Rat Aorta Rings



		rat pancreatic B-cells, % RIS[a]			
compd	R	50 μM	10 μM	1 μM	rat aorta rings ED_{50}[b] (μM)
12a	$CH_2CH_2CH_3$	6.3 ± 0.6 (12)	47.4 ± 3.6 (14)	101.7 ± 4.7 (16)	>300 (4)
12b	$CH(CH_3)_2$[c]	4.4 ± 0.7 (12)	8.5 ± 0.9 (24)	67.6 ± 4.3 (20)	274.0 ± 19.0 (5)
12c	$CH(CH_3)CH_2CH_3$	8.5 ± 1.4 (9)	23.4 ± 1.7 (15)	92.4 ± 5.8 (15)	17.5 ± 3.6 (4)
12d	$CH(CH_2)_3$[c,d]	4.0 ± 0.2 (12)	14.1 ± 0.9 (15)	71.3 ± 3.8 (14)	157.3 ± 7.4 (6)
13a	CH_2CH_3	N.D.	75.0 ± 4.0 (24)	N.D.	162.5 ± 12.8 (4)
13b	$CH(CH_3)_2$	N.D.	65.9 ± 4.4 (14)	N.D.	233.2 ± 29.2 (4)
13c	$CH(CH_2)_3$[d]	N.D.	84.9 ± 4.0 (16)	N.D.	>300 (4)
20a	$CH_2CH_2CH_3$	52.0 ± 2.9 (14)	77.9 ± 4.4 (22)	N.D.	2.9 ± 0.5 (4)
20b	$CH(CH_3)_2$	7.1 ± 1.0 (13)	7.4 ± 0.6 (13)	71.5 ± 4.3 (14)	13.1 ± 3.4 (6)
20c	$CH(CH_3)CH_2CH_3$	28.6 ± 1.8 (15)	76.1 ± 4.2 (22)	N.D.	8.9 ± 2.6 (4)
	diazoxide	26.7 ± 1.6 (16)[c]	73.9 ± 4.4 (16)[c]	87.5 ± 5.0 (15)[c]	22.4 ± 2.1 (11)[c]
	BPDZ 44	8.6 ± 0.9 (23)	13.7 ± 1.2 (23)	70.5 ± 4.4 (19)	154.4 ± 14.5 (8)[c]
	BPDZ 73	5.7 ± 0.5 (35)[c]	4.9 ± 0.4 (32)[c]	36.2 ± 2.4 (31)[c]	36.3 ± 2.2 (6)[c]

[a] RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [c] Published results or previously reported compounds (refs 26, 35, 39). [d] Cyclobutyl.

reduced B-cell activity observed with 3-alkylamino-7-pentyl- and 3-alkylamino-7-ethoxy-4*H*-1,2,4-benzothiadiazine 1,1-dioxides compared to that observed with 7-methyl- and 7-methoxy-substituted compounds (compare **11a**, **11b**, and **11c** with **10a**, **10b**, and **10d**, respectively; compare **13b** and **13c** with **12b** and **12d**, respectively), could be explained at least in part by the increase of the size of the hydrocarbon chain in the 7-position (steric effect).

The rank order of potency, as inhibitors of insulin release, for compounds bearing an isopropylamino side chain in the 3-position appeared to be: 7-COOH compound (**21b**) = 7-COOMe compound (**25**) = 7-C_5H_{11} compound (**12b**) ≤ 7-SO_2CH_3 compound (**31b**) = 7-NH_2 compound (**33**) = 7-$NHCOCH_3$ compound (**34**) < 7-CN compound (**24a**) = 7-$CONH_2$ compound (**26**) ≤ 7-C_2H_5O compound (**13b**) = 7-NO_2 compound (**32b**) < 7-H compound (**22c**) < 7-CF_3 compound (**20b**) = 7-CH_3 compound (**10b**) = 7-CH_3O compound (**12b**) < 7-chloro compound (BPDZ 73) (Tables 1−4).

The effect of the introduction of an electron-withdrawing group in the 7-position remained, however, unclear (i.e. CN **24a** and SO_2CH_3 **31b** compared to CF_3 **20b**). Indeed, the presence of a CN or a SO_2CH_3 group provoked a drastic decrease of activity on insulin-secreting B-cells. Such an effect has already been described in a previous work with 7-NO_2-substituted compounds.[31] Surprisingly, the CF_3 group did not induce

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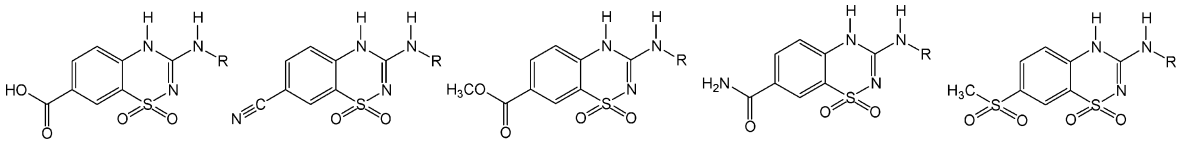
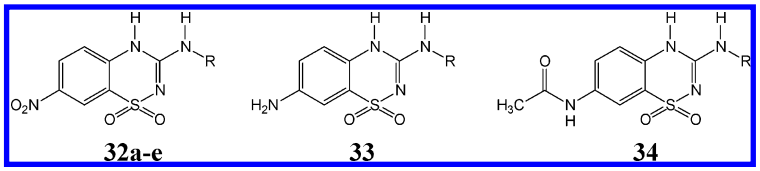


21a-c **24a,b** **25** **26** **31a-c**

		rat pancreatic B-cells % RIS[a]			
compd	R	50 μM	10 μM	1 μM	rat aorta rings ED_{50}[b] (μM)
21a	$CH_2CH_2CH_3$	95.3 ± 3.3 (15)	97.6 ± 6.8 (15)	N.D.	>300 (4)
21b	$CH(CH_3)_2$	98.6 ± 3.5 (16)	96.6 ± 5.0 (23)	N.D.	>300 (4)
21c	$CH(CH_3)CH_2CH_3$	91.2 ± 4.8 (16)	93.0 ± 6.1 (23)	N.D.	>300 (4)
24a	$CH(CH_3)_2$	23.5 ± 1.2 (21)	82.2 ± 5.0 (15)	N.D.	42.7 ± 2.9 (4)
24b	$CH(CH_2)_3$[d]	54.3 ± 4.3 (21)	102.6 ± 8.3 (15)	N.D.	57.9 ± 6.6 (4)
25	$CH(CH_3)_2$	94.3 ± 6.2 (20)	95.7 ± 4.9 (22)	N.D.	>200 (8)
26	$CH(CH_3)_2$	N.D.	76.6 ± 3.7 (23)	N.D.	175.2 ± 35.2 (4)
31a	CH_2CH_3	N.D.	91.6 ± 3.4 (16)	N.D.	>300 (4)
31b	$CH(CH_3)_2$	N.D.	90.5 ± 4.8 (15)	N.D.	127.2 ± 17.9 (4)
31c	$CH(CH_3)\ CH_2CH_3$	N.D.	97.2 ±3.9 (16)	N.D.	126.1 ± 19.5 (4)
	Diazoxide	26.7 ± 1.6 (16)[c]	73.9 ± 4.4 (16)[c]	87.5 ± 5.0 (15)[c]	22.4 ± 2.1 (11)[c]
	BPDZ 44	8.6 ± 0.9 (23)	13.7 ± 1.2 (23)	70.5 ± 4.4 (19)	154.4 ± 14.5 (8)[c]
	BPDZ 73	5.7 ± 0.5 (35)[c]	4.9 ± 0.4 (32)[c]	36.2 ± 2.4 (31)[c]	36.3 ± 2.2 (6)[c]

[a] RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [c] Published results (ref 26). [d] Cyclobutyl.

Table 4. Effects of 7-Substituted 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides on Insulin Secretion from Rat Pancreatic Islets and Contractile Activity of K^+-Depolarized Rat Aorta Rings



32a-e **33** **34**

		rat pancreatic B-cells % RIS[a]			
compd	R	50 μM	10 μM	1 μM	rat aorta rings ED_{50}[b] (μM)
32a	$CH_2CH_2CH_3$	88.9 ± 4.6 (16)[c]	N.D.	N.D.	15.7 ± 3.0 (7)
32b	$CH(CH_3)_2$	4.8 ± 0.6 (14)[c]	64.0 ± 3.8 (37)[c]	N.D.	23.1 ± 3.5 (4)
32c	$CH(CH_3)CH_2CH_3$	63.3 ± 3.8 (18)[c]	N.D.	N.D.	6.1 ± 0.9 (4)
32d	$CH(CH_3)CH(CH_3)_2$	90.4 ±5.2 (16)[c]	N.D.	N.D.	2.8 ± 0.8 (4)
32e	$CH(CH_2)_3$[d]	59.6 ± 4.9 (13)[c]	N.D.	N.D.	13.8 ± 1.6 (4)
33	$CH(CH_3)_2$	85.8 ± 4.3 (15)[c]	N.D.	N.D.	>300 (4)
34	$CH(CH_3)_2$	89.3 ± 5.8 (16)[c]	N.D.	N.D.	>300 (4)
	Diazoxide	26.7 ± 1.6 (16)[c]	73.9 ± 4.4 (16)[c]	87.5 ± 5.0 (15)[c]	22.4 ± 2.1 (11)[c]
	BPDZ 44	8.6 ± 0.9 (23)	13.7 ± 1.2 (23)	70.5 ± 4.4 (19)	154.4 ± 14.5 (8)[c]
	BPDZ 73	5.7 ± 0.5 (35)[c]	4.9 ± 0.4 (32)[c]	36.2 ± 2.4 (31)[c]	36.3 ± 2.2 (6)[c]

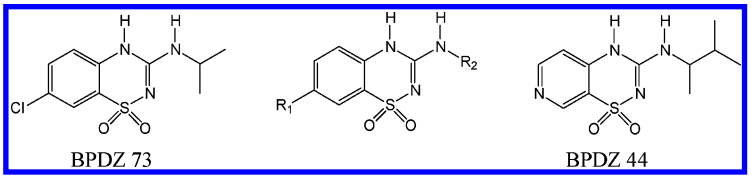
[a] RIS: percentage of residual insulin release from rat pancreatic islets incubated in the presence of 16.7 mM glucose (mean ± SEM (*n*)). [b] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*)). [c] Published results (refs 26 and 31). [d] Cyclobutyl.

any marked reduction of activity on B-cells. The pronounced inhibitory effect of the trifluoromethyl-substituted derivative bearing an isopropylamino side chain in the 3-position (**20b**) may be compared to that of its 7-chloro-substituted counterpart BPDZ 73. Both compounds bear a small size lipophilic substituent in the 7-position which exerts either a moderate (Cl) or pronounced (CF_3) electron-withdrawing effect.[32] By contrast, the CN, SO_2CH_3, and NO_2 substituents represent electron-withdrawing groups which rather increase hydrophilicity. The presence of an electron-donor group such as a methyl (i.e. **10a**, **10b**) or a methoxy moiety (i.e. **12b**, **12d**) appeared to increase activity on the pancreatic tissue (electronic effect). Derivatives having just an hydrogen atom in the 7-position (**22a**−**e**) were found to exhibit, on insulin-secreting cells, an intermediate activity between the electron-withdrawing group (i.e. compounds **21**, **24**, **31**) and the electron-donor group (i.e. compounds **10**, **12**).

The choice of the alkylamino side chains introduced in the 3-position of the 4*H*-benzothiadiazine 1,1-dioxides was inferred from our preliminary investigations with 3-alkylamino-7-halo-4*H*-1,2,4-benzothiadiazine 1,1-dioxides.[26] As expected, the most potent inhibitors of insulin secretion carry a short linear or branched hydrocarbon chain such as ethyl and isopropyl (see **12b**, **20b**, **22a**, **10a**, **10b**). In the same way, the activity of the 3-cyclobutylamino-substituted derivatives (see **10d**, **12d** and **22e**) was close to that of the 3-ethyl (**10a** and **22a**) or 3-isopropylamino-substituted (**10b**, **12b** and **22c**) compounds in their respective series.

The myorelaxant activity of the newly synthesized compounds as well as previously synthesized[31] 7-NO_2, 7-NH_2 and 7-$NHCOCH_3$ compounds was evaluated on

Table 5. Tissue Selectivity Ratio: Pancreatic versus Aortic Tissue for Selected Compounds



compd	R_1	R_2	rat pancreatic B-cells: IC_{50}[a] (μM)	rat aorta rings: ED_{50}[b] (μM)	ED_{50}/IC_{50}[c]
22c	H	$CH(CH_3)_2$	3.8	>300 (4)	>79
10a	CH_3	CH_2CH_3	2.5	210.2 ± 32.2 (6)	84
10b	CH_3	$CH(CH_3)_2$	2.0	200.0 ± 47.6 (3)	100
10c	CH_3	$CH(CH_3)CH_2CH_3$	11.2	15.5 ± 3.2 (10)	1.4
10d	CH_3	$CH(CH_2)_3$[e]	5.6	58.4 ± 7.0 (4)	10
12a	CH_3O	$CH_2CH_2CH_3$	8.9	>300 (4)	>34
12b	CH_3O	$CH(CH_3)_2$	3.7	274.0 ± 19.0 (5)	74
12c	CH_3O	$CH(CH_3)CH_2CH_3$	4.0	17.5 ± 3.5 (4)	4.4
12d	CH_3O	$CH(CH_2)_3$[e]	2.2	157.3 ± 7.4 (6)	72
20b	CF_3	$CH(CH_3)_2$	1.7	13.1 ± 3.4 (6)	7.7
	diazoxide		22.6[d]	22.4 ± 2.1 (11)[d]	1.0
	BPDZ 73		0.7[d]	36.3 ± 2.2 (6)[d]	52
	BPDZ 44		4.4[d]	154.4 ± 14.5 (8)[d]	35

[a] IC_{50}: drug concentration giving 50% inhibition of insulin release (estimated value). [b] ED_{50}: drug concentration giving 50% relaxation of the 30 mM KCl-induced contraction of rat aortic rings (mean ± SEM (*n*). [c] ED_{50}/IC_{50}: estimated selectivity ratio. [d] Published results (refs 26). [e] Cyclobutyl.

K^+-depolarized rat aorta rings (Tables 1 to 4). As previously noted for the pancreatic model, the potency of the drugs was affected by the nature of the hydrocarbon chain on the exocyclic nitrogen atom in the 3-position but also by the nature of the substituent in the 7-position. Except for 7-CF_3-substituted (**20a**−**c**), 7-pentyl-substituted (**11a**,**b**), 7-NO_2-substituted (**32a**−**e**), and 3-*sec*-butylamino-substituted compounds (**10c**, **12c**), most of the 4*H*-1,2,4-benzothiadiazine 1,1-dioxides exhibited a weaker vasorelaxant activity on rat aorta rings than diazoxide. As a rule, the introduction of an electron-withdrawing group (i.e. CF_3, NO_2, CN) rather than an electron-donor group (CH_3, OCH_3) in the 7-position appeared to improve the myorelaxant activity (compare **10b**, **12b**, and **13b** with **20b**, **24a,** and **32b**). 7-COOH-substituted compounds (**21a**−**c**) were found to be inactive, probably due at least in part to the same reason than that mentioned for the pancreatic model (deprotonation at physiological pH). The ester **25**, which cannot be deprotonated at physiological pH, was also inactive, and the amide **26** only exhibited a poor myorelaxant activity. Likewise, the absence of a substituent in the 7-position was responsible for a lack of effect on rat aorta rings. The weak activity of 7-SO_2-CH_3-substituted compounds **31a**−**c** and of the 7-carboxamido compound (**26**) could also be due to the drastic increase of hydrophilicity induced by the substituent introduced in the 7-position.[32]

3-Alkylamino-7-trifluoromethyl- (**20a**−**c**) and 3-alkylamino-7-pentyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxides (**11a**,**b**) were found to express a marked myorelaxant activity on vascular smooth muscle cells. Thus, compared to 7-methyl-substituted compounds **10a**, **b,** and **d**, the introduction of a trifluoromethyl or a pentyl group in the 7-position, which increased the lipophilicity, also improved the myorelaxant effect of the drugs.

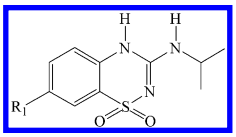
The myorelaxant activity was usually improved with the increase of the size and the branching of the alkylamino chain in the 3-position (see the 3-*sec*-butylamino-substituted compounds **10c** and **12c** compared to the corresponding 3-isopropylamino-substituted compounds **10b** and **12b**). Such a result is in accordance with previous structure−activity relationships reported for cyanoguanidines[33] and pyrido-[24] and benzothiadiazine 1,1-dioxides.[26] Surprisingly, in the 7-pentyl series of compounds (**11a**−**c**), the most potent compound has an ethylamino side chain in the 3-position. However, in this particular series of compounds (**11a**−**c**), and supporting the view that the global steric hindrance and the global lipophilicity of the drugs have to be limited, the presence of a large pentyl group in the 7-position could favor the myorelaxant activity of drug bearing a small alkylamino side chain in the 3-position.

ED_{50}/IC_{50} ratios have been calculated in order to appreciate the apparent tissue selectivity (vascular versus pancreatic tissue) of some selected drugs (see Table 5). Diazoxide, which is known for its lack of tissue selectivity was found to be nearly equipotent on both tissues. BPDZ 73 (**5**), as previously described in the literature,[12,26] was more selective for the pancreatic than the aortic tissue (selectivity ratio = 52).

7-Methyl-substituted and 7-methoxy-substituted 4*H*-1,2,4-benzothiadiazine 1,1-dioxides were found to be the most interesting drugs in terms of potency and selectivity for the pancreatic tissue. Indeed, such compounds, exhibiting an ethylamino (**10a**), an isopropylamino (**10b**, **12b**), or a cyclobutylamino (**10d**, **12d**) side chain in the 3-position, were potent inhibitors of the insulin releasing process while their myorelaxant activity was very less pronounced. The selectivity ratio for **10b** was estimated to be 105. As expected, the loss of selectivity of the 7-Me and 7-MeO-substituted compounds for the pancreatic versus aortic smooth muscle tissue was linked to the increase of the size of the hydrocarbon chain in the 3-position (see **10c** and **12c**, Table 5).

The presence of an hydrogen atom in the 7-position (**22c**) was responsible for a lack of effect on rat aortic ring but appeared to further pancreatic B-cells activity. In contrast, the 7-trifluoromethyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide **20b**, despite of a marked pancreatic

Table 6. pK_a Values of Selected 3-Isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides



compd	R_1	pK_a value[a]
22c	H	9.98
BPDZ 138	F	9.63[b]
BPDZ 73	Cl	9.51[b]
BPDZ 135	Br	9.41[b]
BPDZ 69	I	9.28[b]
20b	CF_3	8.96
24a	CN	8.56
10b	CH_3	10.21
12b	CH_3O	10.11
BPDZ 145	NO_2	7.91[b]
BPDZ 212	NH_2	10.49[b]
BPDZ 213	CH_3CONH	9.80[b]
diazoxide		8.62[b]

[a] The ionization constant was determined by UV spectroscopy in different aqueous buffers of pH ranking from 5 to 12. [b] Published results (refs 26, 31, 34).

activity, exhibited, like diazoxide, a poor tissue selectivity.

Biological results also indicated that the 7-pentyl-substituted compound **11a** exhibited a tissue selectivity (vascular smooth muscle versus pancreatic tissue) opposite to that usually observed with 3-(ethylamino)-substituted compounds (i.e. **22a** and **10a**, Table 1). This finding suggests that a minor structural modification can reverse tissue selectivity.

As previously reported for diazoxide and 3-alkylamino-4*H*-1,2,4-pyridothiadiazine 1,1-dioxides, the acidity of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides is correlated with the presence of a labile proton in the 4-position of the thiadiazine ring.[24,26,34] The presence of this hydrogen atom, and thus the nonionization of the 4-position, appeared to be required for biological activity on the pancreatic tissue.[24,26,34] Using the UV spectrophotometry method, we have determined and compared with previously published values [26,31] the pK_a values (Table 6) of a selection of 4*H*-1,2,4-benzothiadiazine dioxides diversely substituted in the 7-position. When comparing diazoxide (8.69) [34] to BPDZ 73 (9.51), two 7-chloro-substituted benzothiadiazine 1,1-dioxides, it appeared that the introduction of an alkylamino side chain in the 3-position increased the pK_a value (Table 6). This was probably due to the lone pair delocalization of the exocyclic nitrogen atom into the guanidinic system. The pK_a values of previously synthesized halo-substituted compounds (BPDZ 69, BPDZ 73, BPDZ 135, BPDZ 138) showed that the increase of the acidic character (7-F: 9.63, 7-Cl: 9.51, 7-Br: 9.41, and 7-I: 9.28) can be satisfactorily correlated with the increase of the Hammett σ_p constant of the substituent (0.15, 0.24, 0.26, 0.28, respectively; compare to 7-hydrogeno-substituted compound **22c** with $\sigma_p = 0$ and pK_a value of 9.98).[26] As expected, the halo-substituted compounds should exist in more than 99% in the nonionized state at the physiological pH (7.4). Table 6 also reports derivatives bearing electron-withdrawing groups (**20b**, **24a**, BPDZ 145, BPDZ 213) or electron-donating groups (**10b**, **12b**, BPDZ 212) in the 7-position. Derivatives substituted with an electron-donating group were shown to be less acidic than diazoxide and 7-hydrogeno-substituted compound **22c**. They should be mainly nonionized at physiological pH. In contrast, compounds **24a** and BPDZ 145, two compounds bearing an electron-withdrawing group, were more acidic than diazoxide and compound **22c.** The calculated ionized fraction at pH 7.4 was 23.6% for the nitro derivative (BPDZ 145) and 6.5% for the cyano derivative (**24**), respectively. To some extent, the weaker activity of 7-nitro compounds as inhibitors of insulin release, could be explained by a pK_a value lower than 8. In the same context, previously described 3-alkyl-4*H*-1,2,4-pyridothiadiazine 1,1-dioxide with a pK_a value close to 7.5−7.6 were shown to be inactive on the pancreatic tissue.[24]

Taken as a whole, these results confirm that the nonionized form of 3-alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxides is required, but insufficient, for optimal interaction with the receptor (hydrogen bond interaction between the N−H group in the 4-position and the receptor).

Radioisotopic experiments conducted with the pancreatic selective compound **12b** indicated that the drug (50 μM) was able to increase ^{86}Rb (^{42}K substitute) outflow from prelabeled and perifused rat pancreatic islets. Such an effect was completely abolished by the addition of glibenclamide (10 μM) in the medium (data not shown). Those data, as well as previously reported results,[35] confirmed that the 7-methoxy compound expressed the classical pharmacological profile of an ATP-sensitive potassium channel opener.

Conclusions

The presence of an electron-donating group such as a methyl or a methoxy moiety or the absence of substituent in the 7-position of 3-alkylamino-4*H*-1,2,4-benzthiadiazine 1,1-dioxides appeared to be required for increasing activity and selectivity for the pancreatic tissue. The higher selectivity for the pancreatic endocrine tissue was obtained with derivatives bearing an isopropylamino, an ethylamino, or a cyclobutylamino side chain in the 3-position (**10a**, **10b**, **10d**, **12b**, **12d**, **22c**).

In contrast, two series of compounds bearing an electron-withdrawing group such as CF_3 (**20a**−**c**) and NO_2 (**32a**−**e**) or a larger lipophilic hydrocarbon chain in the 7-position (**11a**,**b**) were found to express a marked myorelaxant activity on vascular smooth muscle cells. Compound **11a** even exhibited a tissue selectivity (vascular smooth muscle vs pancreatic tissue) opposite to that usually observed with compounds bearing small groups such as an ethylamino side chain in the 3-position.

Taken as a whole, these results indicated that the modulation of the nature of the substituent in the 7-position can profoundly affect biological activity and tissue selectivity. The present work further indicated that parameters such as lipophilicity, electron-donating or -withdrawing properties and steric effect of the substituent in the 7-position notably influenced the pharmacological profile of the drugs.

Last, radioisotopic experiments conducted on rat pancreatic islets with a typical example of pancreatic B-cell selective drug (**12b**) confirmed that the target of such compounds was the K_{ATP} channel.

The structure−activity relationships deduced from this study will permit to further improve the pharmacophoric model initially proposed for drugs activating the pancreatic K_{ATP} channel.[25,26]

Experimental Section

Chemistry. Melting points were determined on a Büchi-Tottoli capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1000 FT spectrophotometer. The ^{1}H NMR spectra were taken on a Bruker AW-80 (80 MHz) instrument in DMSO-d_6 with HMDSO as internal standard or on a Bruker Avance 500 (500 MHz) instrument in DMSO-d_6 with TMS as internal standard; chemical shifts are reported in δ values (ppm) relative to internal HMDSO or TMS. All reactions were routinely checked by TLC on silica gel Merck 60F 254.

7-Methyl-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (7a). Chlorosulfonyl isocyanate (16.8 mL, 193.24 mmol) and nitromethane (160 mL) were mixed together in a closed dried vessel. The mixture was cooled at −5 °C (ice-salt bath) and protected from moisture during the slow addition, under vigorous stirring, of 4-toluidine (17.8 g, 166.12 mmol) dissolved in nitromethane (50 mL). When the addition was completed, anhydrous $AlCl_3$ (28.0 g, 210.1 mmol) was added to the resulting suspension and the mixture was refluxed for 30 min. The hot solution was poured onto ice (800 g) and, after stirring and the complete melting of ice, the resulting precipitate was collected by filtration and washed with water (75 mL). The insoluble crude material was suspended in an aqueous solution of $NaHCO_3$ (10 g/200 mL) and heated until most of the precipitate was solubilized. The suspension was treated with charcoal, filtered, and the filtrate was adjusted to pH 1 with 6 N HCl. The resulting white product was collected by filtration, washed with water, and dried. (19.38 g, 55%); mp 298−302 °C (lit.: 316−318 °C [27]).

7-Pentyl-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (7b). The title compound was obtained from 4-pentylaniline (27.01 g, 166.00 mmol) by following the experimental conditions described for **7a**, except that the crude material was dissolved in a hydromethanolic 1:1 solution of $NaHCO_3$ (30 g/600 mL). (23.16 g, 55%); mp > 300 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{12}H_{16}N_2O_3S$) C, H, N, S.

7-Methoxy-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (7c). The title compound was obtained from 4-methoxyaniline (20.44 g, 166.00 mmol) by following the experimental conditions described for **7a** (17.05 g, 45%); mp 275−277 °C (lit.: 300 °C[27]).

7-Ethoxy-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (7d). The title compound was obtained from 4-ethoxyaniline (22.77 g, 166.00 mmol) by following the experimental conditions described for **7a** (20.11 g, 50%); mp 243−244 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_9H_{10}N_2O_4S$) C, H, N, S.

7-Methyl-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (8a). The mixture of 7-methyl-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **(7a)** (20.3 g, 95.65 mmol) and phosphorus pentasulfide (40 g, 179.95 mmol) in anhydrous pyridine (250 mL) was heated under reflux for 5 h. The resulting suspension was concentrated under reduced pressure and the residue was dissolved in the minimum of 2 N NaOH. This solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 1 with 6 N HCl. The crystalline solid was collected by filtration, washed with water, and dried (14.75 g, 65%); mp 220−224 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_8H_8N_2O_2S_2$) C, H, N, O, S.

7-Pentyl-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (8b). The title compound was obtained as described for **8a** starting from **7b** (25.7 g, 95.65 mmol) (16.32 g, 60%); mp 198−202 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{12}H_{16}N_2O_2S_2$) C, H, N, S.

7-Methoxy-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (8c). The title compound was obtained as described for **8a** starting from **7c** (22 g, 95.15 mmol). The reflux was maintained during 3 h. (19.78 g, 66%); mp 219−222 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_8H_8N_2O_3S_2$) C, H, N, S.

7-Ethoxy-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-Dioxide (8d). The title compound was obtained as described for **8a** starting from **7d** (28 g, 115.03 mmol). The reflux was maintained during 3 h. (22.87 g, 77%); mp 223−225 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_9H_{10}N_2O_3S_2$) C, H, N, S.

7-Methyl-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (9a). 7-Methyl-3-thioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide **(8a)** (12.0 g, 52.63 mmol) was dissolved in an aqueous solution of $NaHCO_3$ (7 g/280 mL). Under stirring, methanol (300 mL) and methyl iodide (12 mL, 192.34 mmol) were successively added to this solution. After 30 min under stirring at room temperature, the mixture was adjusted to pH 3 with 1 N HCl and methanol was removed under reduced pressure. After cooling, the title product was collected by filtration, washed with water and dried (10.93 g, 85%); mp 275−278 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_9H_{10}N_2O_2S_2\cdot H_2O$) C, H, N, S.

3-Methylsulfanyl-7-pentyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (9b). The title compound was obtained as described for **9a** starting from **8b** (12.0 g, 42.19 mmol) (10.70 g, 85%); mp 237−241 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{13}H_{18}N_2O_2S_2$) C, H, O, S.

7-Methoxy-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (9c). The title compound was obtained as described for **9a** starting from **8c** (12.0 g, 49.12 mmol) (11.80 g, 93%); mp 270−276 °C, IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_9H_{10}N_2O_3S_2\cdot H_2O$) C, H, N, S.

7-Ethoxy-3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (9d). The title compound was obtained as described for **9a** starting from **8d** (12.0 g, 46.45 mmol) (9.94 g, 78%); mp 243−248 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{10}H_{12}N_2O_3S_2$) C, H, N, S.

2-Chloro-5-trifluoromethylbenzenesulfonamide (15). A suspension of 2-chloro-5-trifluoromethylbenzenesulfonyl chloride (**14**, 10.0 g, 38.4 mmol) in an aqueous solution of ammonia (10% w/v, 150 mL) was stirred at room temperature. After 1 h, the solution was treated with charcoal, the filtrate was concentrated by an half and adjusted to pH 1 with 6 N HCl. The precipitate was collected by filtration, washed with water and dried (8.97 g, 90%); mp 153−155 °C (Lit.: 158.5−160 °C[36]).

2-Benzylamino-5-trifluoromethylbenzenesulfonamide (16). A solution of 2-chloro-5-trifluoromethylbenzenesulfonamide (**15**, 3 g, 11 mmol) in benzylamine (30 mL) was heated at 100 °C. Most of the amine was removed by distillation under reduced pressure and the residue was suspended in water (30 mL). An aqueous (5% w/v) solution of NaOH was added (30 mL). The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 2 with 6 N HCl. The precipitate was collected by filtration, washed with water and dried (3.05 g, 80%); mp 125−127 °C; IR (KBr);^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{14}H_{13}F_3N_2O_2S$) C, H, N, S.

3-Oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-carboxylic acid 1,1-Dioxide Monohydrate (17). 7-Methyl-3-oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7a**, 5 g, 23.56 mmol) was suspended in water (500 mL) and dissolved by addition of 2.5 N NaOH. $KMnO_4$ (12 g, 75.95 mmol) was added portionwise, and the resulting mixture was stirred at 70 °C for 3 h. The insoluble material was filtered off, and $NaHSO_3$ was added to the filtrate until the pink coloration disappeared. The solution was treated with charcoal and filtered. The filtrate was adjusted to pH 1 with 12 N HCl. The precipitate was collected by filtration, washed with water, and dried (3.76 g, 66%); mp > 300 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_8H_6N_2O_5S\cdot H_2O$) C, H, N, S.

2-Amino-5-trifluoromethylbenzenesulfonamide (18a). A solution of 2-benzylamino-5-trifluoromethylbenzenesulfonamide (**16**, 2 g, 6 mmol) in methanol (20 mL) was supplemented with Pd/C (0.2 g, 5%). The mixture was poured in a

hermetically closed autoclave under hydrogen atmosphere (5 bar). The insoluble material was filtered off and the solvent removed under reduced pressure. The residue was dissolved in a minimum aqueous NaOH solution (10% w/v), treated with charcoal and filtered, and the filtrate was adjusted to pH 1 with 6 N HCl. The precipitate was collected by filtration, washed with water and dried (1.27 g, 85%); mp 142−142 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_7H_7F_3N_2O_2S$) C, H, N, S.

4-Amino-3-sulfamoylbenzoic Acid (18b). 3-Oxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-carboxylic acid 1,1-dioxide (**17**, 5 g, 20.64 mmol) was suspended in an aqueous solution of sulfuric acid (50% w/v) and stirred under reflux. When the insoluble has disappeared, the solution was cooled to 0 °C and supplemented with an aqueous solution of NaOH (30% w/v) until pH 2. The precipitate was collected by filtration, washed with water, and dried (4.06 g, 91%); mp 214−219 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_7H_8N_2O_4S$) C, H, N, S.

3-(1*H*-Imidazol-1-yl)-7-trifluoromethyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (19a). A solution of 2-amino-5-trifluoromethylbenzenesulfonamide (**18a**, 5.5 g, 22 mmol) and *N*,*N*′-thiocarbonyldiimidazole (14.0 g, 78,22 mmol) in dioxane (55 mL) was refluxed for 4 h. The solvent was removed by distillation under reduced pressure, and the residue was suspended in water (150 mL). A solution of NaOH in water (2.5 g/20 mL) was added, and the solution was stirred at room temperature for 10 min. The alkaline solution was treated with charcoal and filtered. The filtrate was adjusted to pH 2 with 6 N HCl. The precipitate was collected by filtration, washed with water,, and dried. (4.87 g, 70%); mp 211−214 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{11}H_7F_3N_4O_2S$) C, H, N, S.

3-(1*H*-Imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine-7-carboxylic Acid 1,1-Dioxide (19b). The title compound was obtained as described for **19a** starting from **18b** (5.5 g, 25.44 mmol) (5.1 g, 68%); mp > 300 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{11}H_8N_4O_4S$) C, H, N, S.

3-(1*H*-Imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (19c). The title compound was obtained as described for **19a** starting from 2-aminobenzenesulfonamide (5.5 g, 31.94 mmol) (6.26 g, 79%); mp 246−248 °C (Lit.: 246−248 °C [37]); IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{10}H_8N_4O_2S$) C, H, N, S.

2-Chloro-5-methylsulfonylbenzenesulfonamide (28). Chlorosulfonic acid (25 mL) was cooled on an ice-salt bath and then carefully supplemented with 4-chloro-1-methylsulfonylbenzene (**27**, 5 g, 26.22 mmol). The mixture was heated under reflux for 1 h and thionyl chloride (1.5 mL) was added. After 2 h reflux, the reaction mixture was cooled and poured on ice (50 g) under stirring. The resulting precipitate was collected by filtration and washed with cold water. The solid was suspended under stirring in an aqueous solution of ammonia (150 mL, 10 w/v). After 30 min, the alkaline suspension was concentrated under reduced pressure up to a volume of 75 mL. The precipitate was collected by filtration, washed with water, and dissolved in an aqueous solution of NaOH (50 mL, 10 w/v). The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 5 with 6 N HCl. The crystalline precipitate was collected by filtration washed with water, and dried. (3.54 g, 50%); mp 225−227 °C (Lit.: 227−229[39]); Anal. ($C_7H_8ClNO_4S_2$) C, H, N, S.

2-Amino-5-methylsulfonylbenzenesulfonamide (29). The mixture of 2-chloro-5-methylsulfonylbenzenesulfonamide (**28**, 5 g, 18.54 mmol) in a saturated aqueous solution of ammonia (50 mL) was heated in a sealed vessel at 140 °C for 2 h. After cooling, the solution was concentrated to a small volume (20 mL), and the resulting mixture was adjusted to pH 5 with 6 N HCl. The crystalline precipitate was collected by filtration, washed with water, and dried. (4.17 g, 90%); mp 209−210 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_7H_{10}N_2O_4S_2$) C, H, N, S.

***N*-(2-Amino-5-methylsulfonylbenzenesulfonyl)-*N*′-ethylthiourea (30a).** K_2CO_3 (0.85 g, 6.2 mmol) was added to a solution of 2-amino-5-methylsulfonylbenzenesulfonamide (**29**, 1.25 g, 5.0 mmol) in dry acetone (20 mL) under stirring at room temperature. Ethyl isothiocyanate (0.58 mL, 6.6 mmol) was added dropwise, and the resulting mixture was stirred at gentle reflux for 4 h. The cooled mixture was evaporated to dryness under reduced pressure. The residue was dissolved in water (25 mL). The pH was adjusted to 6 with 6 N HCl, and the solution was stirred at 0 °C until the product solidified. The precipitate was collected by filtration, washed with water, and dried (1.01 g, 60%); mp 148−150 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{10}H_{15}N_3O_4S_3$) C, H, N, S.

***N*-(2-Amino-5-methylsulfonylbenzenesulfonyl)-*N*′-isopropylthiourea (30b).** The title compound was obtained as described for **30a** using isopropyl isothiocyanate (0.62 g, 6.1 mmol) (1.13 g, 60%); mp 149−151 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{11}H_{17}N_3O_4S_3$) C, H, N, S.

***R*/S-*N*-(2-Amino-5-methylsulfonylbenzenesulfonyl)-*N*′-2-butylthiourea (30c).** The title compound was obtained as described for **30a** using *R*/S-2-butyl isothiocyanate (0.66 g, 6.1 mmol) (1.28 g, 68%); mp 144−146 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{12}H_{19}N_3O_4S_3$) C, H, N, S.

General Procedure for the Synthesis of the 7-Substituted 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides 10a−d, 11a−c, 12a−d, and 13a−c. The mixture of the appropriate 7-substituted 3-methylsulfanyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide intermediate (**9a−d)** (0.5 g) and the appropriate alkylamine was heated in a sealed vessel for 4 h at 140 °C. The excess of amine was eliminated by distillation under reduced pressure, and the residue was suspended in water (20 mL). An aqueous solution of NaOH (5% w/v) was added dropwise until dissolution of the residue. The alkaline solution was treated with charcoal and filtered. The filtrate was adjusted to pH 4−5 with 6 N HCl. The precipitate was collected by filtration, washed with water, and dried. The compound was recrystallized in the appropriate solvent.

3-Ethylamino-7-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (10a). The title compound was obtained from **9a** (0.5 g) following the general procedure using an aqueous solution of ethylamine 70% (10 mL). Yield: 0.35 g (70%); mp 250−252 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{10}H_{13}N_3O_2S$) C, H, N, S.

3-Isopropylamino-7-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (10b). The title compound was obtained from **9a** (0.5 g) following the general procedure using isopropylamine (5 mL). Yield: 0.40 g (76%); mp 266−268 °C IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{15}N_3O_2S$) C, H, N, S.

***R*/*S*-3-(2′-Butyl)amino-7-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (10c).** The title compound was obtained from **9a** (0.5 g) following the general procedure using *R*/*S*-2-butylamine (5 mL). Yield: 0.35 g (63%); mp 219−220 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{12}H_{17}N_3O_2S$) C, H, N, S.

3-Cyclobutylamino-7-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (10d). The title compound was obtained from **9a** (0.5 g) following the general procedure using a solution of cyclobutylamine (1 mL) in dioxane (5 mL). Yield: 0.43 g (79%); mp 289−290 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{12}H_{15}N_3O_2S$) C, H, N, S.

3-Ethylamino-7-pentyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (11a). The title compound was obtained from **9b** (0.5 g) following the general procedure using an aqueous solution of ethylamine 70% (10 mL).Yield: 0.35 g, (70%); mp 202−205 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{14}H_{21}N_3O_2S$) C, H, N, S.

3-Isopropylamino-7-pentyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (11b). The title compound was obtained from **9b** (0.5 g) following the general procedure using isopropylamine (5 mL). Yield: 0.34 g (65%); mp 198−202 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{15}H_{23}N_3O_2S$) C, H, N, S.

3-Cyclobutylamino-7-pentyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (11c). The title compound was obtained from **9b** (0.5 g) following the general procedure using a solution of cyclobutylamine (1 mL) in dioxane (5 mL). Yield: 0.35 g (66%);

mp 239−241 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{16}H_{23}N_3O_2S$) C, H, N, S.

7-Methoxy-3-propylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (12a). The title compound was obtained from **9c** (0.5 g) following the general procedure using propylamine (5 mL). Yield: 0.42 g (78%); mp 194−199 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{15}N_3O_3S\cdot H_2O$) C, H, N, S.

3-Isopropylamino-7-methoxy-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (12b). The title compound was obtained from **9c** following the general procedure using a solution of isopropylamine (5 mL). Yield: 0.39 g (76%); mp 227−233 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{15}N_3O_3S$) C, H, N, S.

***R*/*S*-3-(2′-Butylamino)-7-methoxy-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (12c).** The title compound was obtained from **9c** (0.5 g) following the general procedure using R/S-2-butylamine (5 mL). Yield: 0.38 g (70%); mp 197−202 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{12}H_{17}N_3O_3S$) C, H, N, S.

3-Cyclobutylamino-7-methoxy-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (12d). The title compound was obtained from **9c** (0.5 g) following the general procedure using a solution of cyclobutylamine (1 mL) in dioxane (5 mL). Yield: 0.32 g (60%); mp 249−252 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{12}H_{15}N_3O_3S$) C, H, N, S.

7-Ethoxy-3-(ethylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (13a). The title compound was obtained from **9d** (0.5 g) following the general procedure using an aqueous solution of ethylamine 70% (10 mL). Yield: 0.38 g (78%); mp 243−248 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz).Anal. ($C_{11}H_{15}N_3O_3S$) C, H, N, S.

7-Ethoxy-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (13b). The title compound was obtained from **9d** (0.5 g) following the general procedure using isopropylamine (5 mL). Yield: 0.32 g (62%); mp 211−215 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz).Anal. ($C_{12}H_{17}N_3O_3S$) C, H, N, S.

3-Cyclobutylamino-7-ethoxy-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (13c). The title compound was obtained from **9d** (0.5 g) following the general procedure using a solution of cyclobutylamine (1 mL) in dioxane (5 mL). Yield: 0.31 g (58%); mp 246−251 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz).Anal. ($C_{13}H_{17}N_3O_3S$) C, H, N, S.

General Procedure for the Synthesis of the 7-Substituted 3-Alkylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxides 20a−c, 21a−c, and 22a−e. The mixture of the appropriate 7-substituted 3-(1*H*-imidazol-1-yl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxide intermediate (**19a**−**c**, 0.5 g) and the appropriate alkylamine (5 mL) was heated in a hermetically closed autoclave at 140 °C for 5 h. After cooling, the excess of the amine was removed by distillation under reduced pressure, and the residue was suspended in water (20 mL). An aqueous solution of NaOH (5% w/v) was added dropwise until dissolution of the residue. The alkaline solution was treated with charcoal and filtered, and the filtrate was adjusted to pH 4−5 (pH 2 for **21a**−**c**) with 6 N HCl. The precipitate was collected by filtration, washed with water, and dried. The compound was purified by crystallization in an appropriate solvent.

3-Propylamino-7-trifluoromethyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (20a). The title compound was obtained from **19a** (0.5 g) following the general procedure using propylamine (5 mL). Yield: 0.26 g, (54%); mp 241−243 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{12}F_3N_3O_2S$) C, H, N, S.

3-Isopropylamino-7-trifluoromethyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (20b). The title compound was from **19a** (0.5 g) following the general procedure using isopropylamine (5 mL). Yield: 0.32 g (66%); mp 287−289 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{12}F_3N_3O_2S$) C, H, N, S.

***R*/*S*-3-(2′-Butylamino)-7-trifluoromethyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (20c).** The title compound was obtained from **19a** (0.5 g) following the general procedure using *R*/*S*-2-butylamine (5 mL). Yield: 0.34 g (68%); mp 234−236 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{12}H_{14}F_3N_3O_2S$) C, H, N, S.

3-Propylamino-4*H*-1,2,4-benzothiadiazine-7-carboxylic Acid 1,1-Dioxide (21a). The title compound was obtained from **19b** (0.5 g) following the general procedure using propylamine (5 mL). Yield: 0.34 (71%); mp 300 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{13}N_3O_4S$) C, H, N, S.

3-isopropylamino-4*H*-1,2,4-benzothiadiazine-7-carboxylic Acid 1,1-Dioxide (21b). The title compound was from **19b** (0.5 g) following the general procedure using isopropylamine (5 mL). Yield: 0.32 g (66%); mp >300 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{13}N_3O_4S$) C, H, N, S.

***R*/*S*-3-(2′-butylamino)-4*H*-1,2,4-benzothiadiazine-7-carboxylic Acid 1,1-Dioxide (21c).** The title compound was obtained from **19b** (0.5 g) following the general procedure using (*R*/*S*)-2-butylamine (5 mL). Yield: 0.34 g (68%); mp >300 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{12}H_{15}N_3O_4S$) C, H, N, S.

3-Ethylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (22a). The title compound was obtained from **19c** (0.5 g) following the general procedure using an aqueous solution of ethylamine 70% (10 mL). Yield: 0.27 g (60%); mp 227−232 °C (lit.:232−233 °C [38]); IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_9H_{11}N_3O_2S$) C, H, N, S.

3-Propylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Monohydrate (22b). The title compound was obtained from **19c** (0.5 g) following the general procedure using propylamine (5 mL). Yield: 0.36 g (74%); mp 190−196 °C (lit.: 195 °C [38]); IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{10}H_{13}N_3O_2S\cdot H_2O$) C, H, N, S.

3-Isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (22c). The title compound was obtained from **19c** (0.5 g) following the general procedure using isopropylamine (5 mL). Yield: 0.36 g (74%); mp 213-218 °C (lit.: 222−223 °C [38]); IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{10}H_{13}N_3O_2S$) C, H, N, S.

***R*/*S*-3-(2-Butylamino)-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (22d).** The title compound was obtained from **19c** (0.5 g) following the general procedure using (*R*/*S*)-2-butylamine (5 mL). Yield: 0.35 g (68%); mp 179−181 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{15}N_3O_2S$) C, H, N, S.

3-Cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (22e). The title compound was obtained from **19c** (0.5 g) following the general procedure using a solution of cyclobutylamine (1 mL) in dioxane (5 mL). Yield: 0.30 g (60%); mp 239−242 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{13}N_3O_2S$) C, H, N, S.[39]

7-Cyano-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (24a). 7-Iodo-3-isopropylamino-4*H*-1,2,4-benzothiadiazine 1,1-dioxide[26] (**23a**, 1.2 g, 3.28 mmol) and CuCN (0.6 g, 6.76 mmol) were suspended in anhydrous DMF (10 mL) under nitrogen, and the mixture was heated under reflux for 5 h. The mixture was supplemented with water (30 mL). The resulting suspension was adjusted to pH 12 by means of an aqueous solution of NaOH (10% w/v), and the alkaline solution was treated with charcoal and filtered. The filtrate was adjusted to pH 1 with 6 N HCl. The precipitate was collected by filtration, washed with water, dried, and recrystallized from hot methanol. Yield: 0.44 g (51%); mp > 300 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{12}N_4O_2S$) C, H, N, S.

7-Cyano-3-cyclobutylamino-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (24b). The title compound was obtained as described for **24a** starting from 3-cyclopropylamino-7-iodo-4*H*-1,2,4-benzothiadiazine 1,1-dioxide[26] (**23b**, 1.2 g, 3.18 mmol). Yield: 0.48 g (55%); mp 299−303 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{12}H_{12}N_4O_2S$) C, H, N, S.

Methyl 3-isopropylamino-4*H*-1,2,4-benzothiadiazine-7-carboxylate 1,1-Dioxide (25). 3-Isopropylamino-4*H*-1,2,4-benzothiadiazine-7-carboxylic acid 1,1-dioxide (**21b**, 0.35 g, 1.23 mmol) was suspended in a solution of H_2SO_4 (0.28 mL) and methanol (3.5 mL), and the mixture was heated under reflux for 4 h. The solvent was removed under reduced pressure. The residue was poured into water (20 mL). The

resulting suspension was adjusted to pH 8 by means of an aqueous solution of NaOH (10% w/v). The precipitate was collected by filtration, washed with water, dried, and recrystallized from hot methanol. Yield: 0.14 g (41%); mp 230−236 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{12}H_{15}N_3O_4S$) C, H, N, S.

3-Isopropylamino-4*H*-1,2,4-benzothiadiazine-7-carboxamide 1,1-Dioxide Monohydrate (26). 3-Isopropylamino-4*H*-1,2,4-benzothiadiazine-7-carboxylic acid 1,1-dioxide (**21b**, 0.35 g, 1.23 mmol) and carbonyldiimidazole (0.22 g, 1.35 mmol) were solubilized in DMF (4 mL), and the mixture was heated for 2 h at 60 °C. The mixture was added under stirring to an aqueous solution of ammonia (5 mL, 10% w/v). After 30 min., the suspension was adjusted to pH 5 with 6 N HCl. The crystalline precipitate was collected by filtration washed with water, and dried. Yield: 0.17 g (48%), mp > 280 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 500 MHz). Anal. ($C_{11}H_{14}N_4O_3S\cdot H_2O$) C, H, N, S.

3-Ethylamino-7-methylsulfonyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (31a). A solution of phosgene (0.52 mL) in toluene (20%) was added dropwise to a solution of *N*-(2-amino-5-methylsulfonylbenzenesulfonyl)-*N*′-ethylthiourea (**30a**, 1 g, 2.9 mmol) and triethylamine (1 mL, 7.1 mmol) in dry THF (30 mL). The solution was stirred at 0 °C for 1 h. The solvent was removed under reduced pressure. The residue was poured into water (20 mL) and stirred for 40 min. The crude product was isolated by filtration, washed with water, dried, and recrystallized from MeOH/water. Yield: 0.54 g (60%); mp 264−270 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{10}H_{13}N_3O_4S_2$) C, H, N, S.

3-Isopropylamino-7-methylsulfonyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (31b). The title compound was obtained as described for **31a** starting from *N*-(2-Amino-5-methylsulfonylbenzenesulfonyl)-*N*′-isopropylthiourea (**30b**, 1 g, 2.8 mmol). Yield: 0.58 g (65%); mp 272−276 °C IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{11}H_{15}N_3O_4S_2$) C, H, N, S.

***R*/*S*-3-(2-Butylamino)-7-methylsulfonyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (31c).** The title compound was obtained as described for **31a** starting from *N*-(*R*/*S*)−(2-Amino-5-methylsulfonylbenzenesulfonyl)-*N*′-2-butylthiourea (**30c**, 1 g, 2.7 mmol). Yield: 0.60 g (66%); mp 244−245 °C; IR (KBr); ^{1}H NMR (DMSO-d_6, 80 MHz). Anal. ($C_{12}H_{17}N_3O_4S_2$) C, H, N, S.

Ionization Constants. The p*K*a values of the compounds were determined by means of UV spectrophotometry using a Perkin-Elmer UV/Vis 554 spectrophotometer at 25 °C. UV spectra of compounds were taken in different aqueous buffers of pH ranking from 5 to 12. The pK_a values were calculated by the Debye−Hückel equation at the maximum basic form absorbance.[40]

Biological Assays. Measurements of Insulin Release from Incubated Rat Pancreatic Islets. Pancreatic islets were isolated by collagenase method from fed Wistar rats (180−220 g). Groups of 10 islets, each derived from the same batch of islets, were preincubated for 30 min at 37 °C in 1 mL of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) dialyzed albumin (Sigma) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%).

The islets were then incubated at 37 °C for 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, the reference compound or the benzothiadiazine derivative. The release of insulin was measured radioimmunologically using rat insulin as a standard.[41] Residual insulin release was expressed as a percentage of the value recorded in control experiment (100%), i.e., in the absence of drug and presence of 16.7 mM glucose.

Measurement of the Contractile Activity in Rat Aorta. All experiments were performed with aorta removed from fed Wistar rats (180−200 g). A section of the aorta was cleared of adhering fat and connective tissue and was cut into transverse rings (3−4 mm long). The endothelium was removed by rubbing the intimal surface with forceps. The segments were suspended under 1.5 g of tension by means of steel hooks in an organ bath containing 20 mL of a Krebs-bicarbonate-buffered solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5. The physiological solutions were maintained at 37 °C and bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The isometric contractions of the aortic rings were measured with a force-displacement transducer. After 60 min of equilibration, the rings were exposed to 30 mM KCl. When the tension had stabilized, the drugs were added to the bath at increasing concentrations until maximal relaxation (or until 0.3 mM). The relaxation response was expressed as the percentage of the contractile response to KCl. The ED_{50} value (drug concentration evoking 50% inhibition of the plateau phase induced by KCl) were assessed from dose curves using Datanalyst software (EMKA Technologies, France).

Measurement of ^{86}Rb Outflow from Rat Pancreatic Islets. The method used for measuring ^{86}Rb (^{42}K substitute) outflow from prelabeled and perifused rat pancreatic islets was previously described in detail.[12,23]

Supporting Information Available: Elemental analyses of the newly synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0311339